Tapping into new applications using automated protein mass spectrometry

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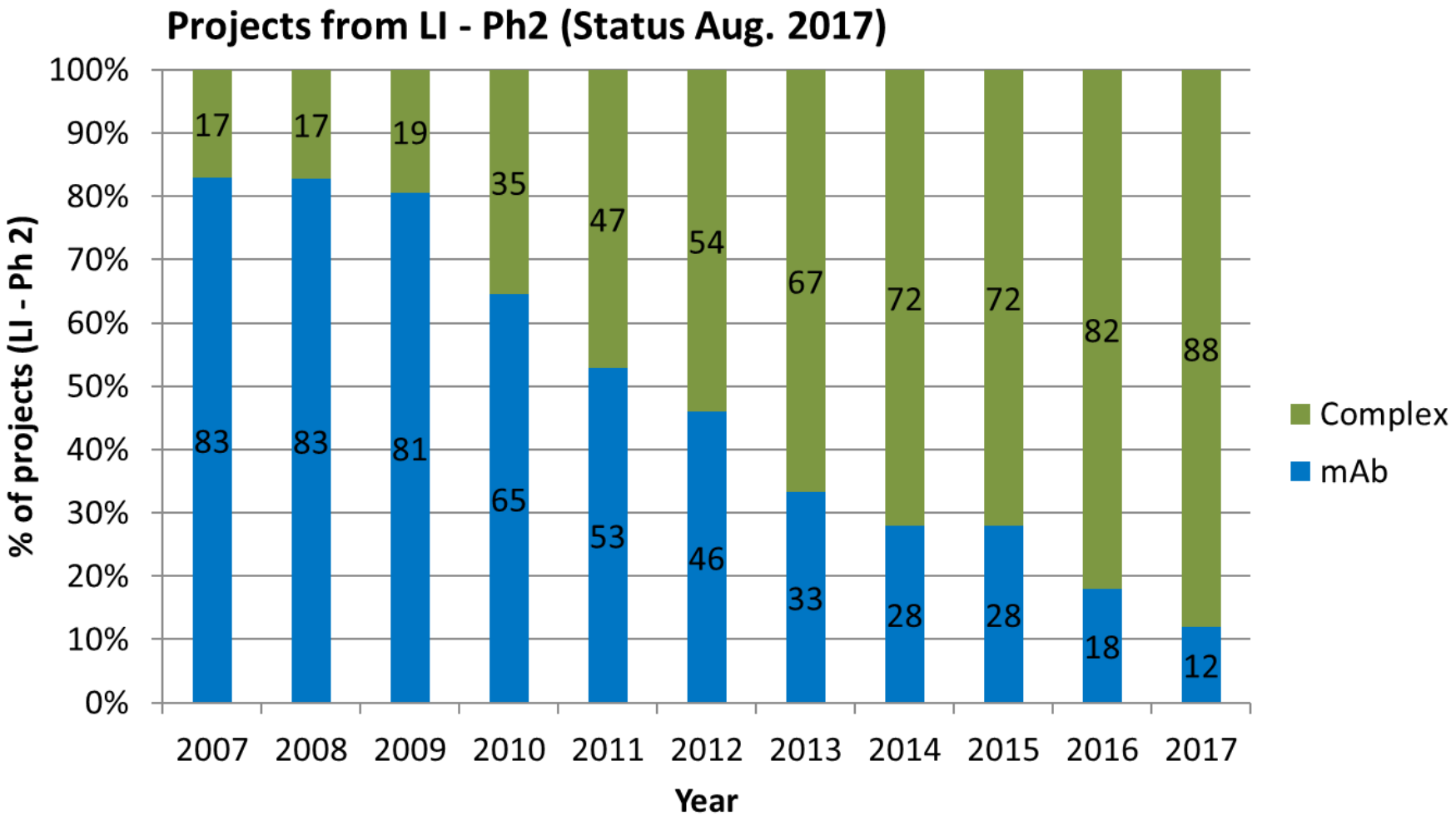
Roche pRED

Outline

- Introduction
- Overview of automated protein mass spectrometry (MS)
- Automation of intact protein MS for screening workflows
- Outlook / next steps
- Summary and acknowledgements

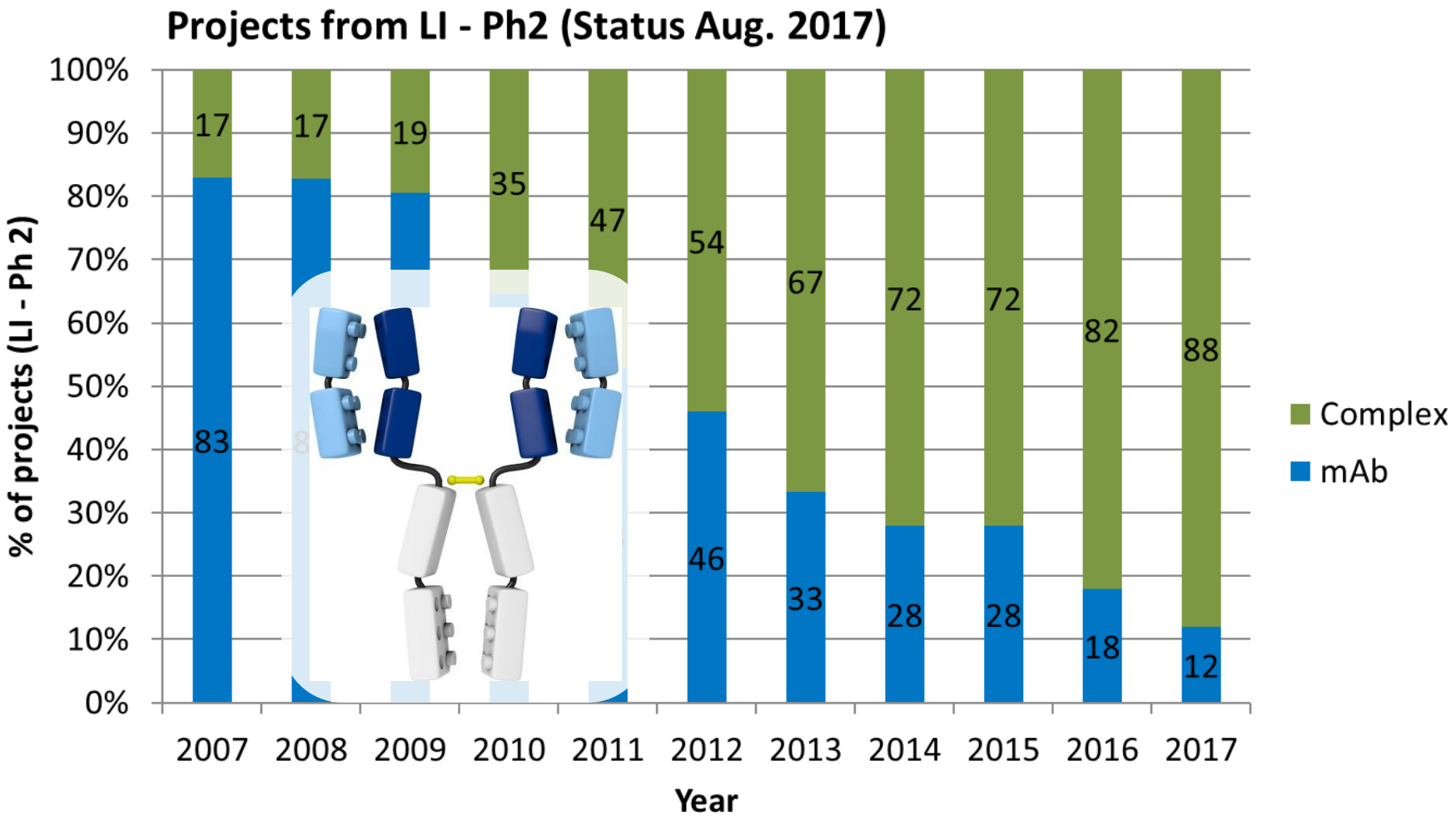
Advancing biotherapeutics

Fuelling the pRED pipeline with complex protein formats



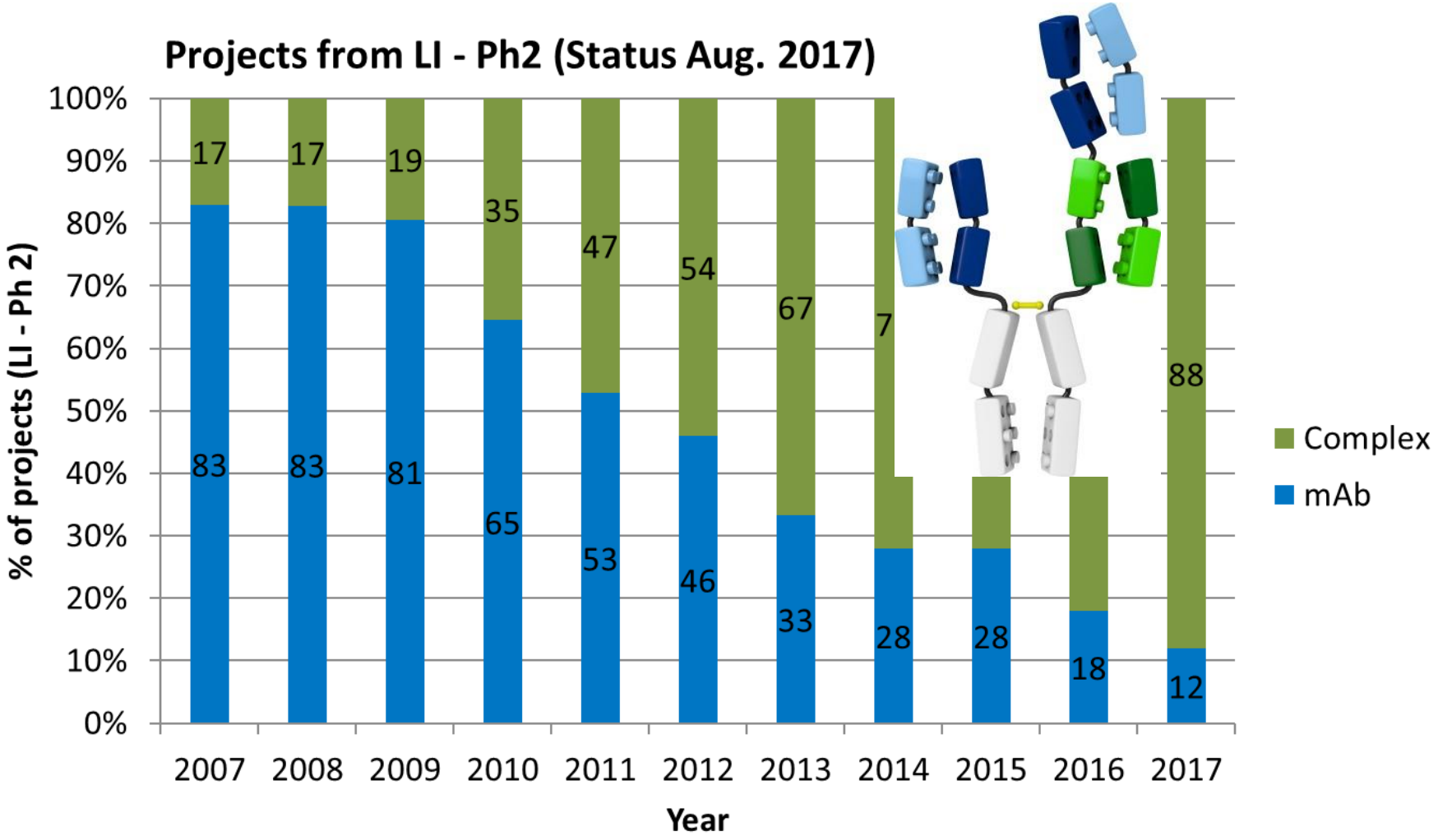
Advancing biotherapeutics

Fuelling the pRED pipeline with complex protein formats



Advancing biotherapeutics

Fuelling the pRED pipeline with complex protein formats



Benefit of hitting more than one target

Binding two targets can induce T-cell activation against cancer cells.

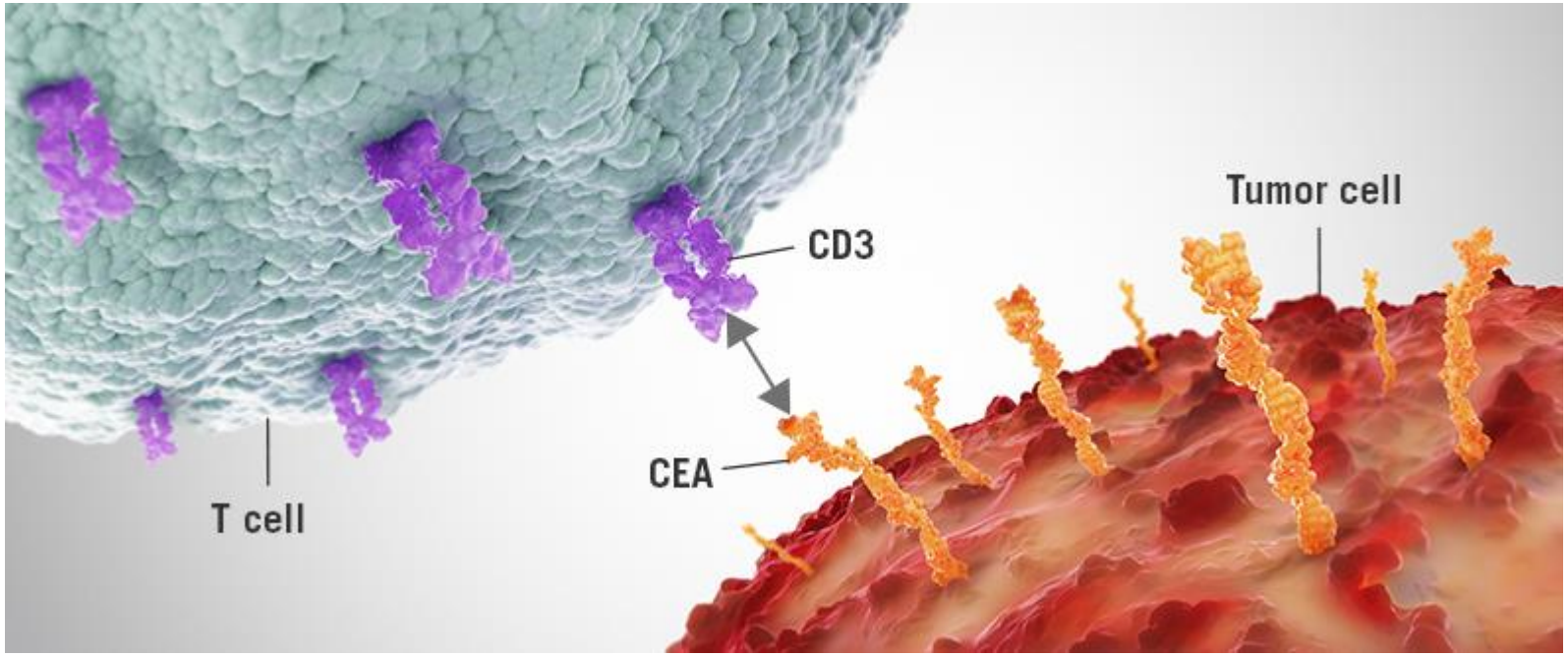
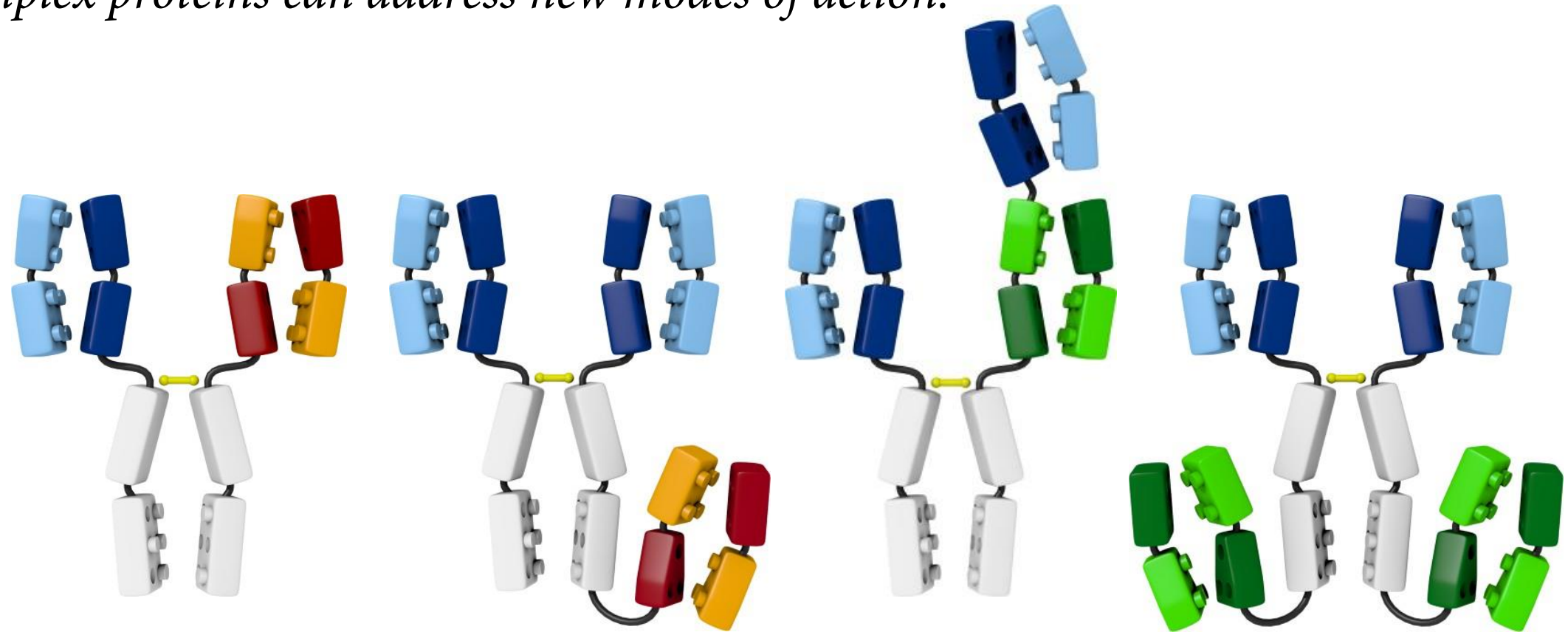


Image source: https://www.biooncology.com/pathways/cancer-immunology/ceacd3.html

Optimizing bispecific biotherapeutics

Complex proteins can address new modes of action.



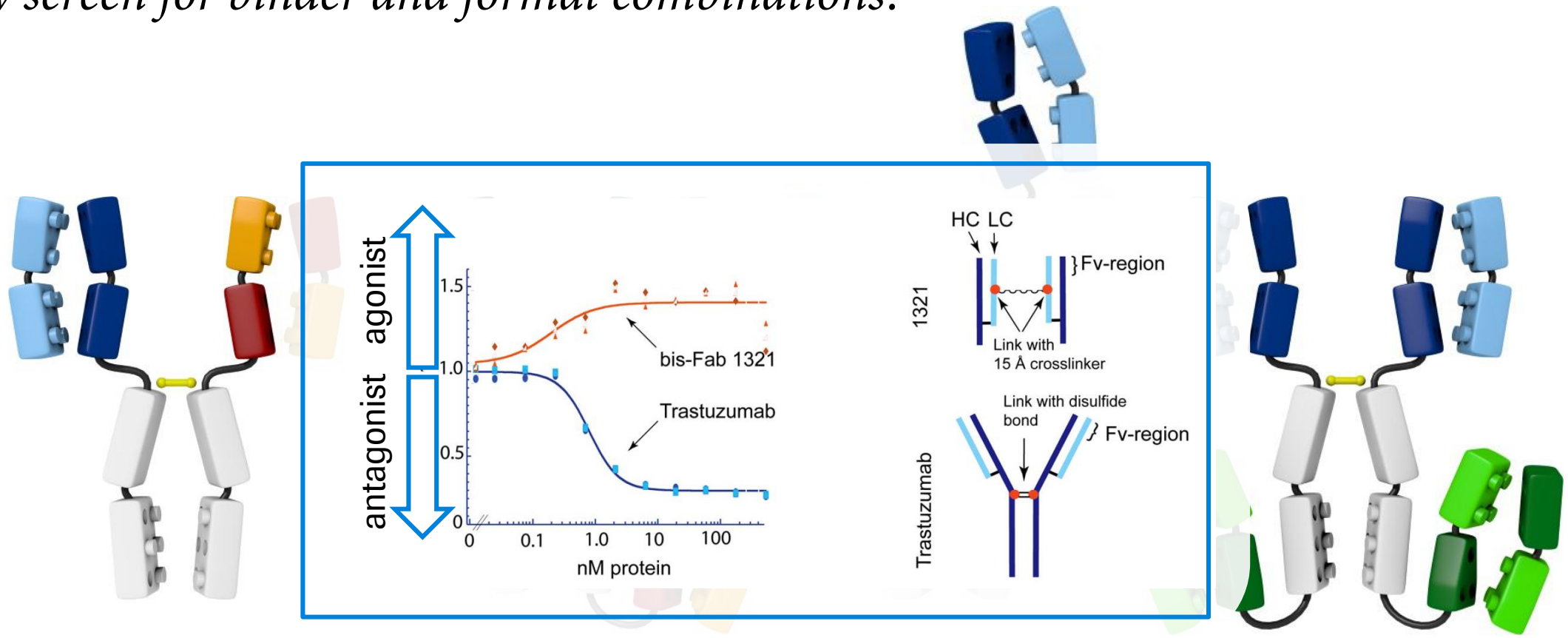
Affinity-avidity profile and mode of action is influenced by protein format.

Scheer et al. Plos One 2012; https://doi.org/10.1371/journal.pone.0051817

Image source: Alex Bujotzek, Computational Engineering and Data Science, pRED

Optimizing bispecific biotherapeutics

Why screen for binder and format combinations?

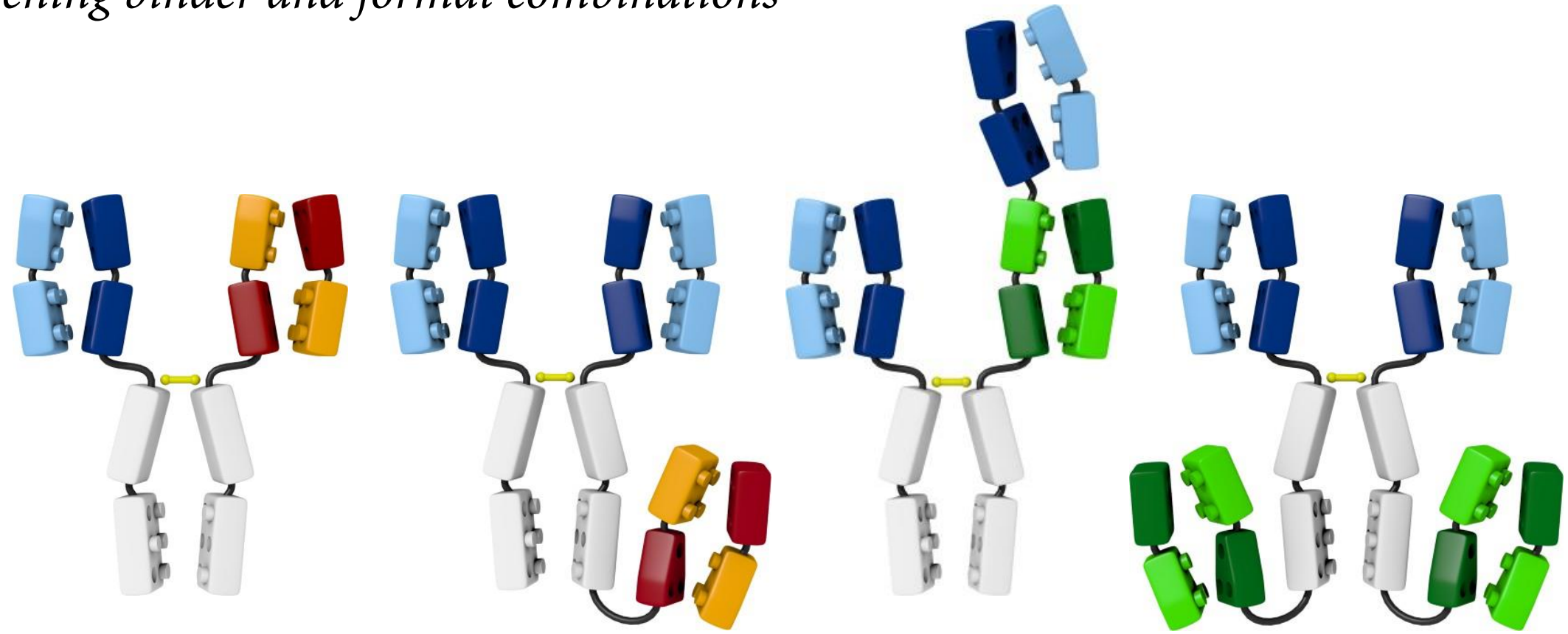


Mode of action even of one single binder can be significantly altered by the protein format used.

Scheer et al. Plos One 2012; https://doi.org/10.1371/journal.pone.0051817

Optimizing bispecific biotherapeutics

Screening binder and format combinations

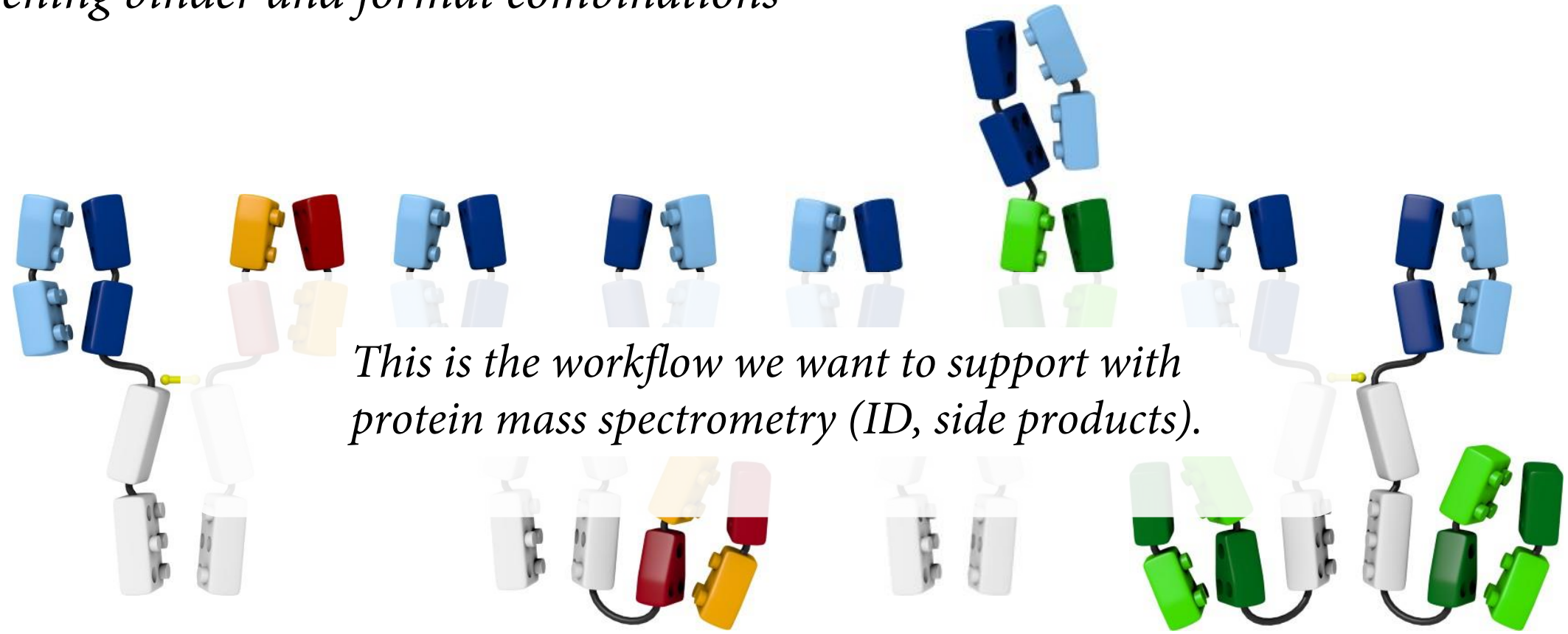


10 binders target A x **10** binders target B x **4** protein formats = **400** different bispecific protein samples

providing a variety of main product masses and covering a broad range of side product profiles

Optimizing bispecific biotherapeutics

Screening binder and format combinations



This is the workflow we want to support with protein mass spectrometry (ID, side products).

10 binders target A x **10** binders target B x **4** protein formats = **400** different bispecific protein samples

providing a variety of main product masses and covering a broad range of side product profiles

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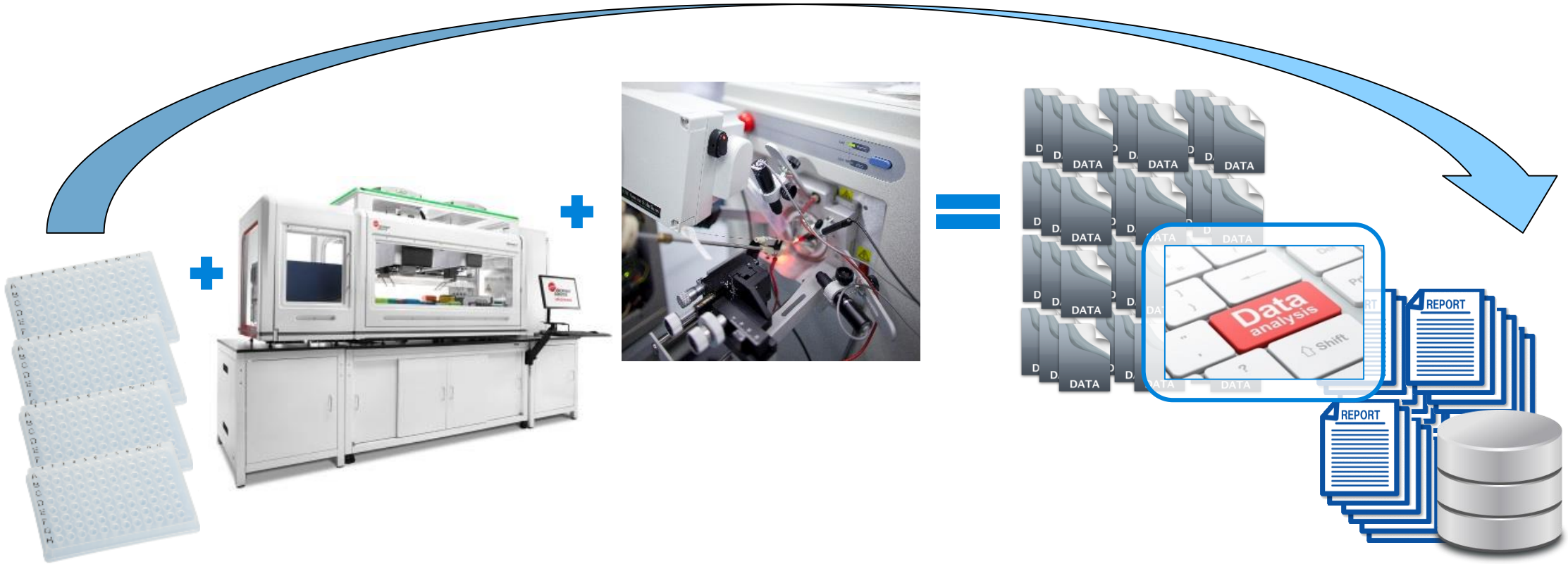
Automation in mass spectrometry: sample-to-report

Leaving details aside …



Samples — Sample preparation — LC-MS experiment — Data evaluation — Reports

Automation in mass spectrometry: sample-to-report

Leaving details aside, but not for too long.

Q1: How much or how little analyst involvement do we need for 400 different protein samples?

Q2: Is there a suitable IT landscape in place to support the associated data workflow?

Samples — Sample preparation — LC-MS experiment — Data evaluation — Reports

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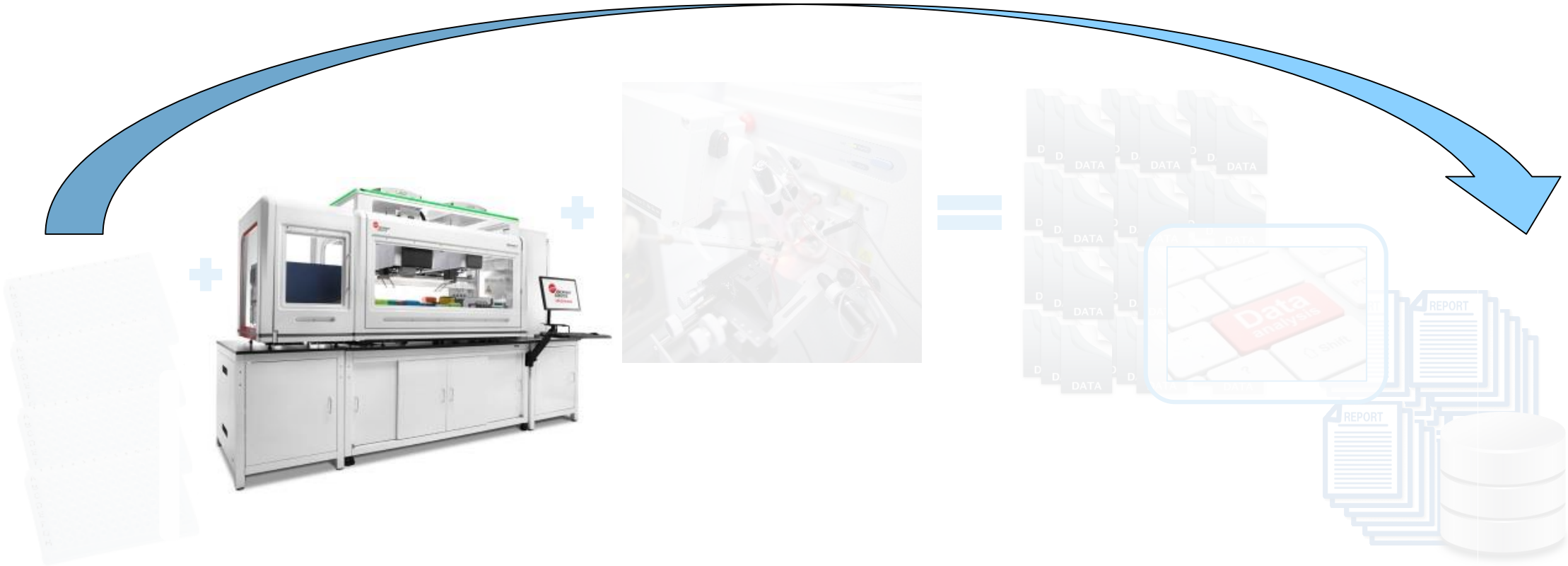
Automation in mass spectrometry: sample-to-report

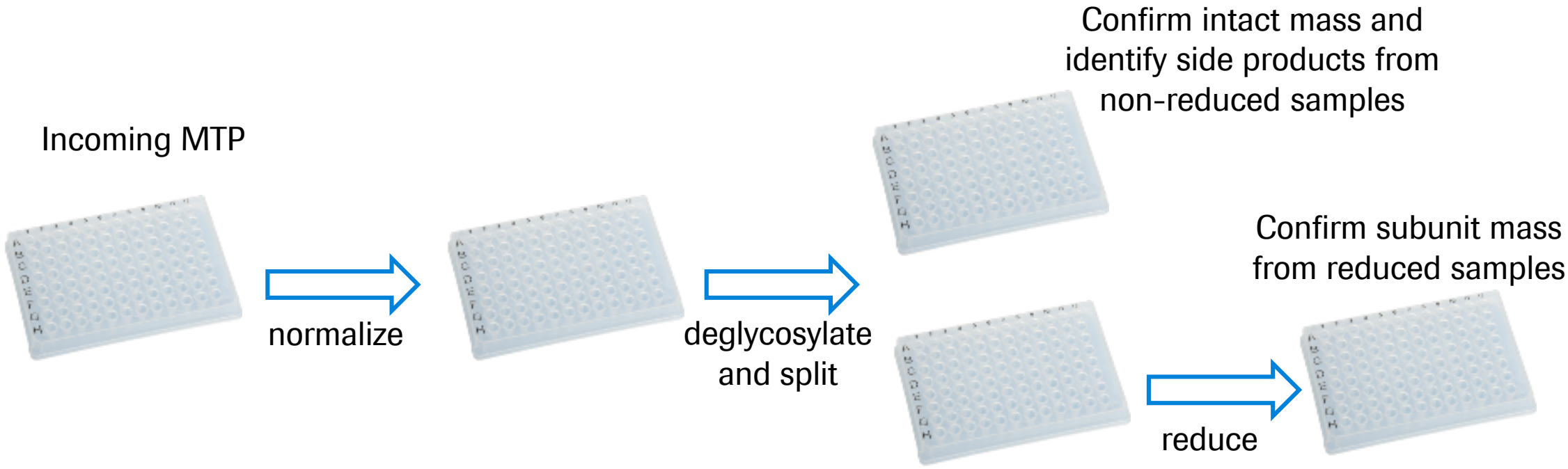
Get started with …



Samples | **Sample preparation** | LC-MS experiment | Data evaluation | Reports

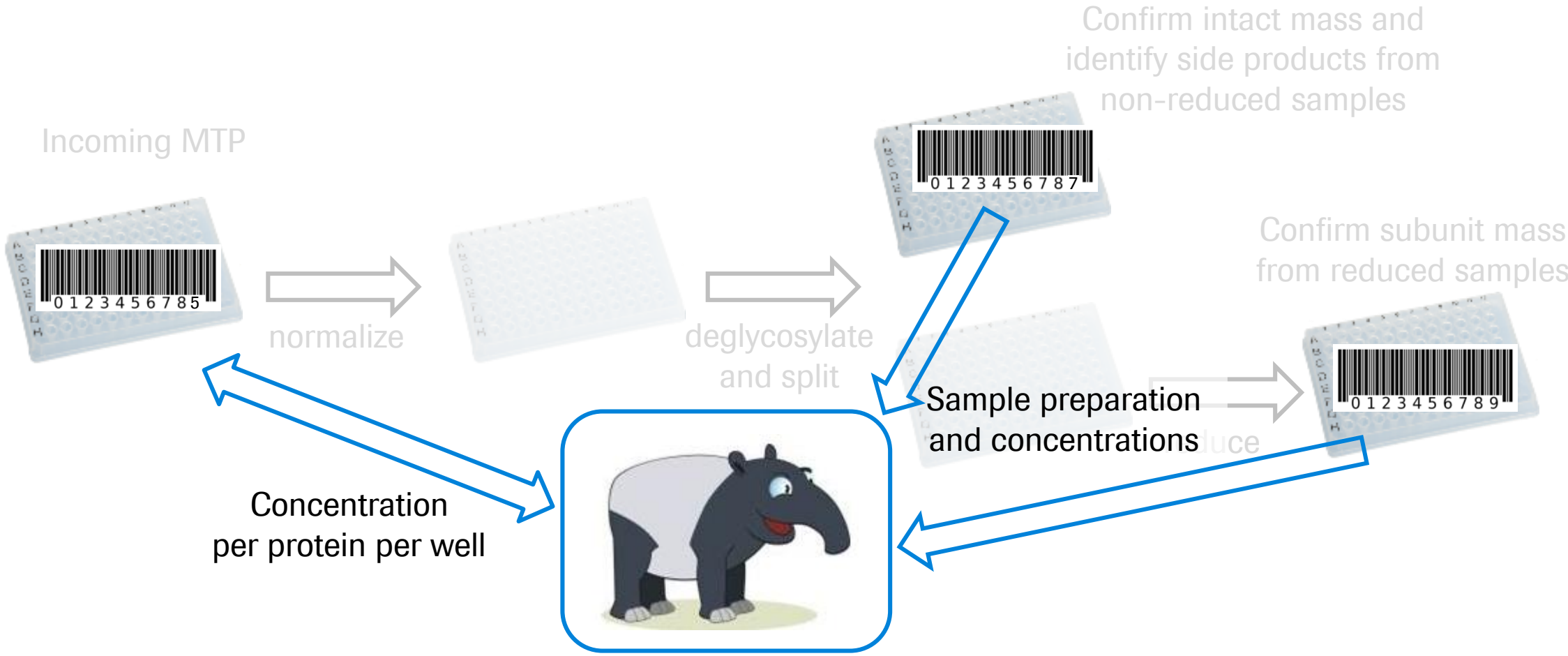
Automating sample preparation

Generic protocols for deglycosylation and reduction are applicable.



Automating sample preparation

Sample data must be retrieved and stored throughout sample preparation.



TaPIR = Therapeutic Protein Identification and Registration System

Automating sample preparation

Sample data must be retrieved and stored throughout sample preparation.

Incoming MTP

normalize

deglycosylate and split

Confirm intact mass and identify side products from non-reduced samples

reduce

Confirm subunit mass from reduced samples

Real world

Digital world

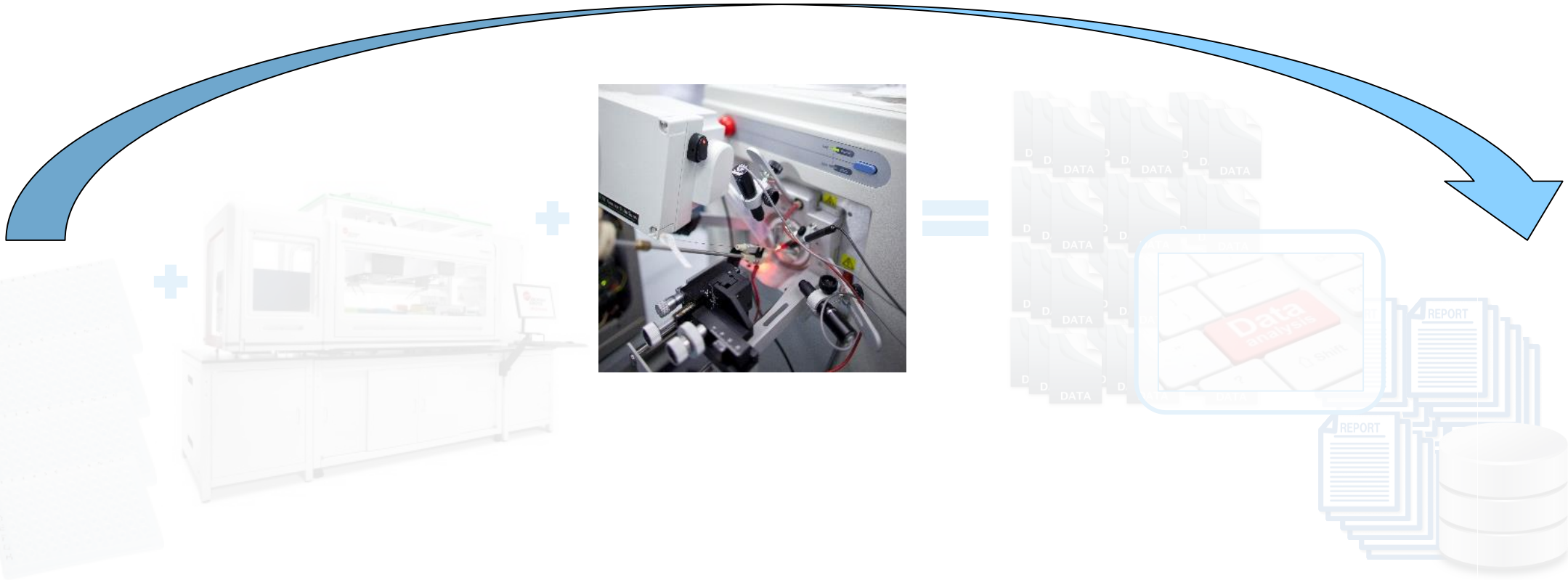
Protein data (amino acid sequences, sample concentrations, …)

Plate info

0123456789

Automation in mass spectrometry: sample-to-report

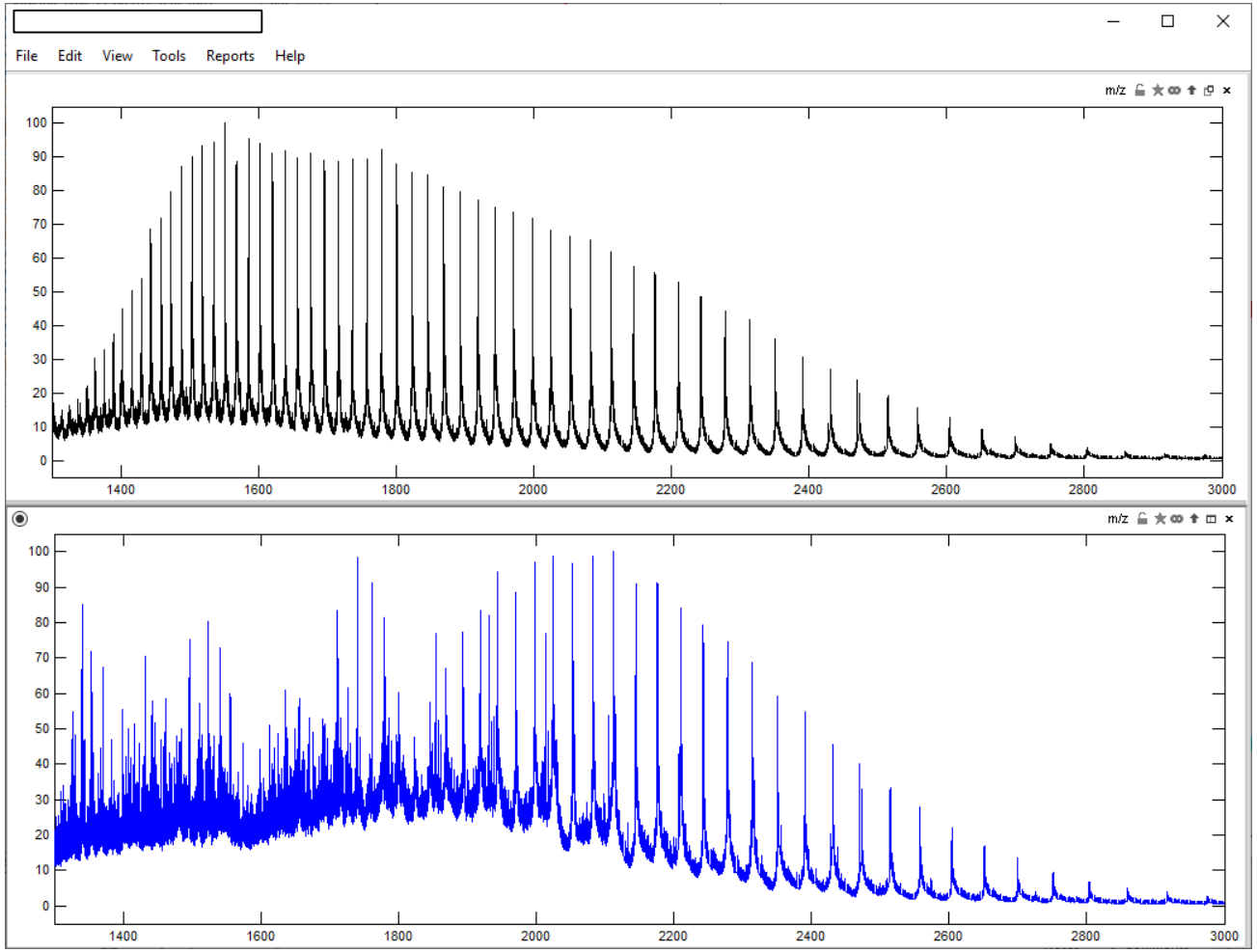
Dive into data acquisition …



Samples | Sample preparation | LC-MS experiment | Data evaluation | Reports

Automating LC–MS experiment setup

Establish format-specific ESI parameters for suitable sample ionization.

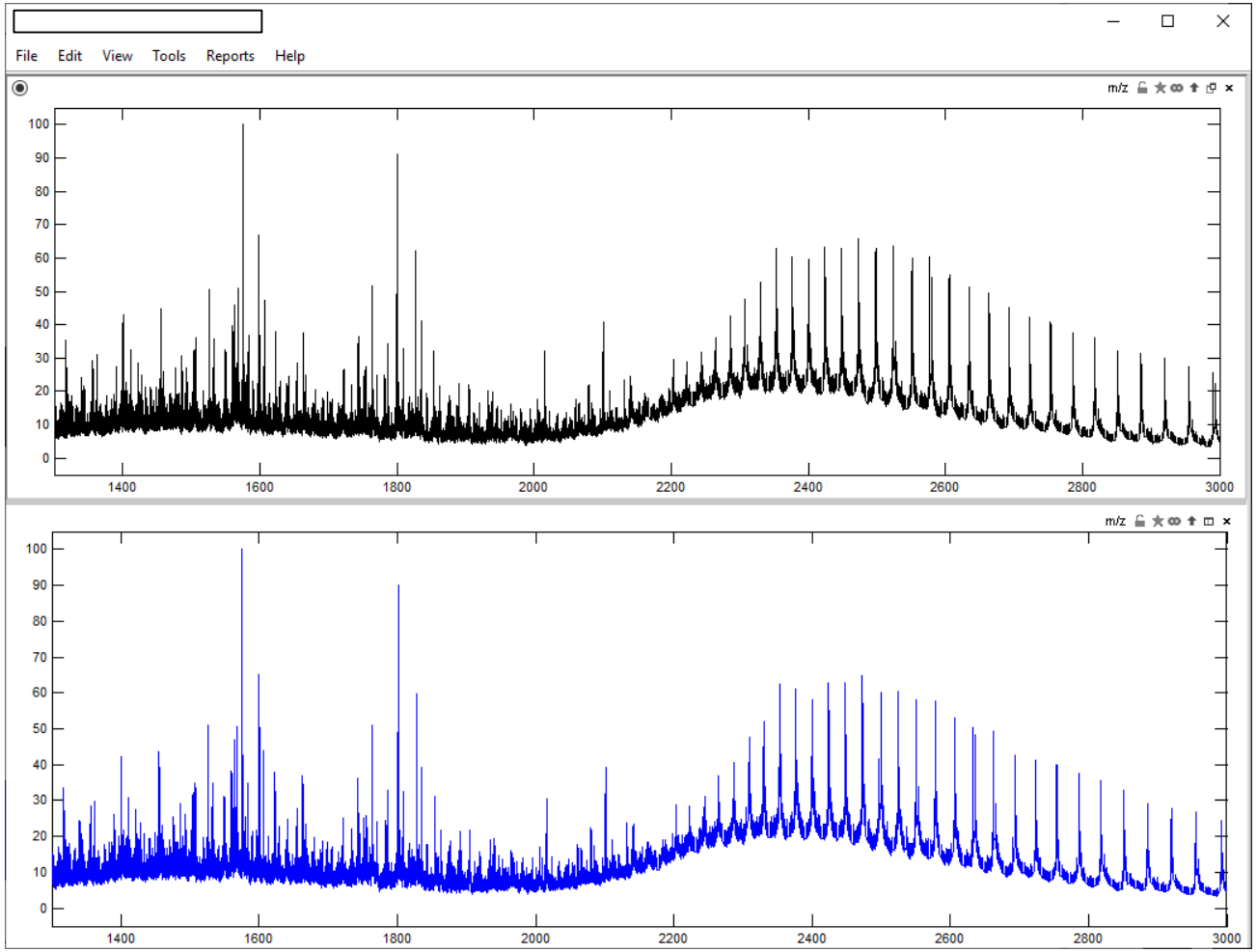


Top/bottom: same sample

Ion source voltages differ

Automating LC–MS experiment setup

Format-specific ESI parameters may be hard to find (no one-size-fits-all).



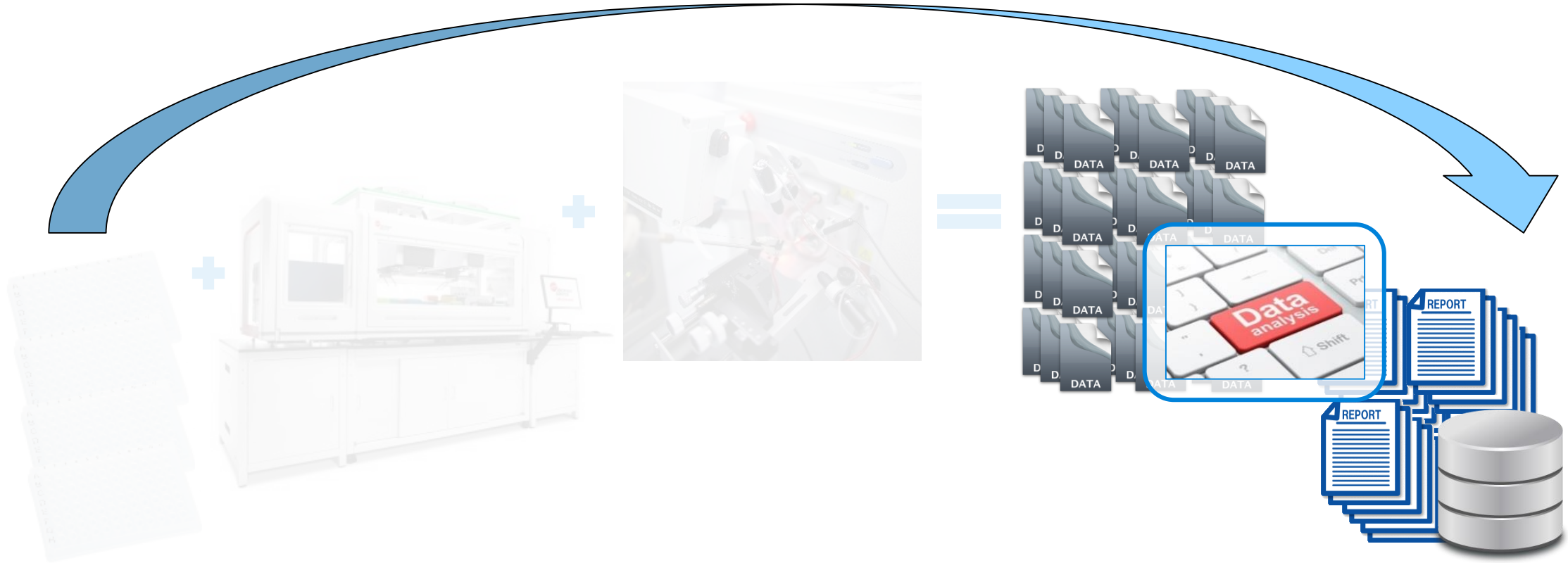
Top/bottom: same sample

Ion source voltages differ

using values as on previous slide

Automation in mass spectrometry: sample-to-report

Deal with the data deluge.

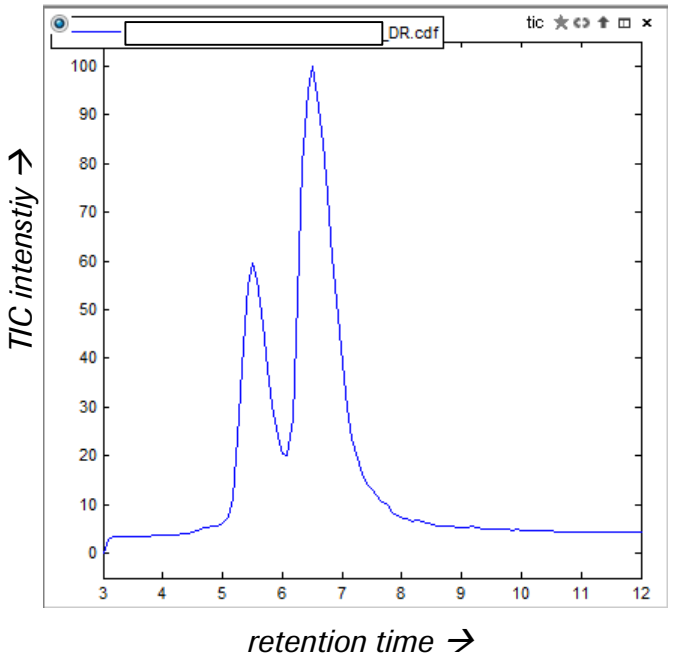


Samples | Sample preparation | LC-MS experiment | **Data evaluation** | Reports

Automating MS data evaluation

Introducing Roche's virtual MS analyst …

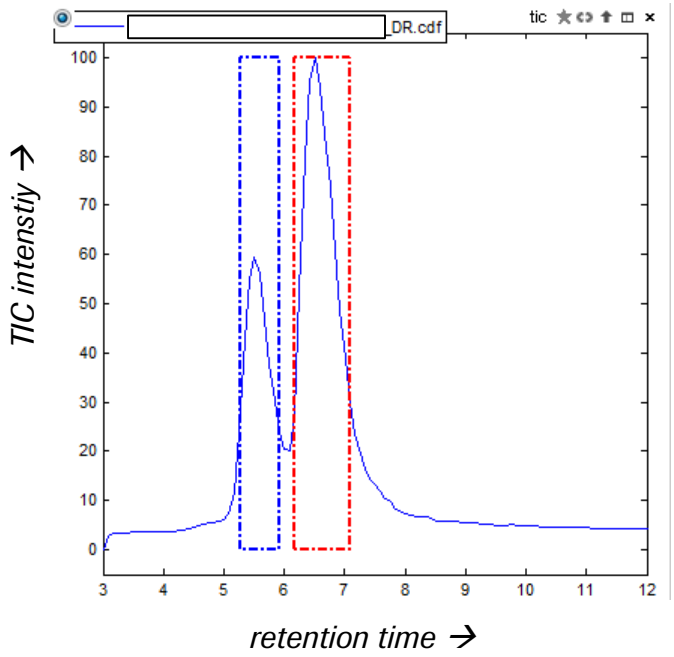
File name contains sample ID and preparation info



Start data evaluation as soon as experiment is finished.

Automating MS data evaluation

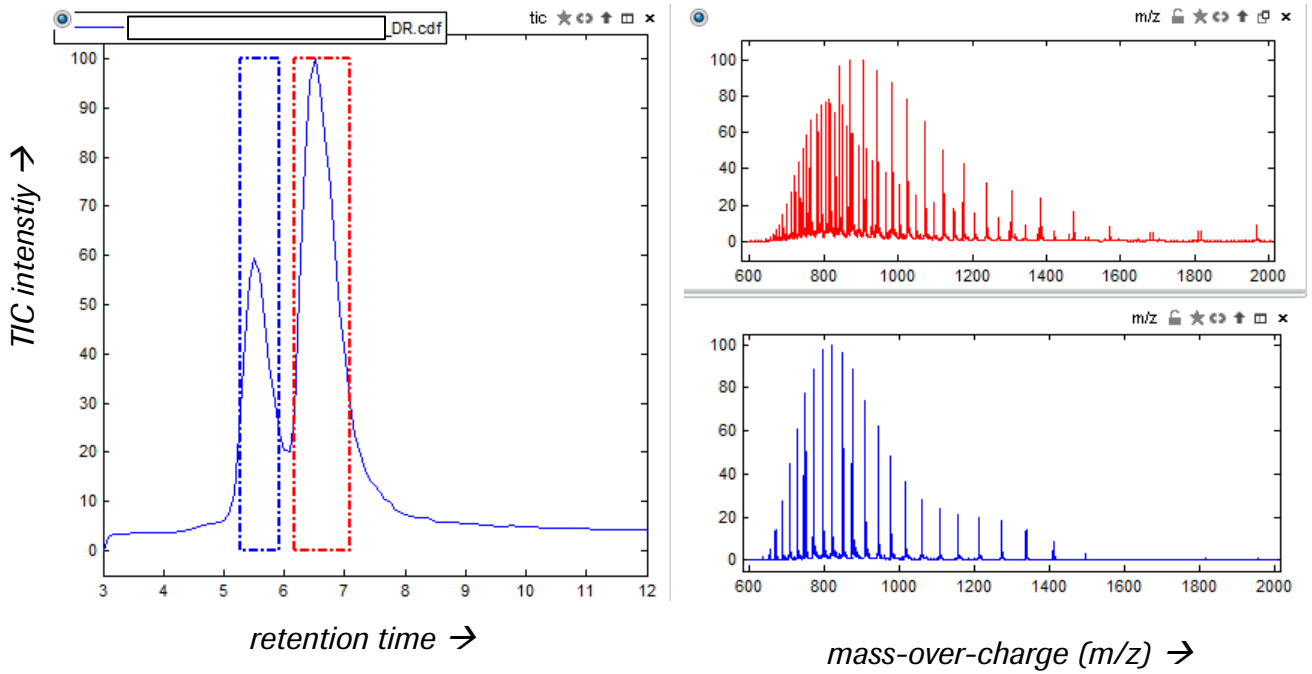
Introducing Roche's virtual MS analyst …



Identify TIC peaks.

Automating MS data evaluation

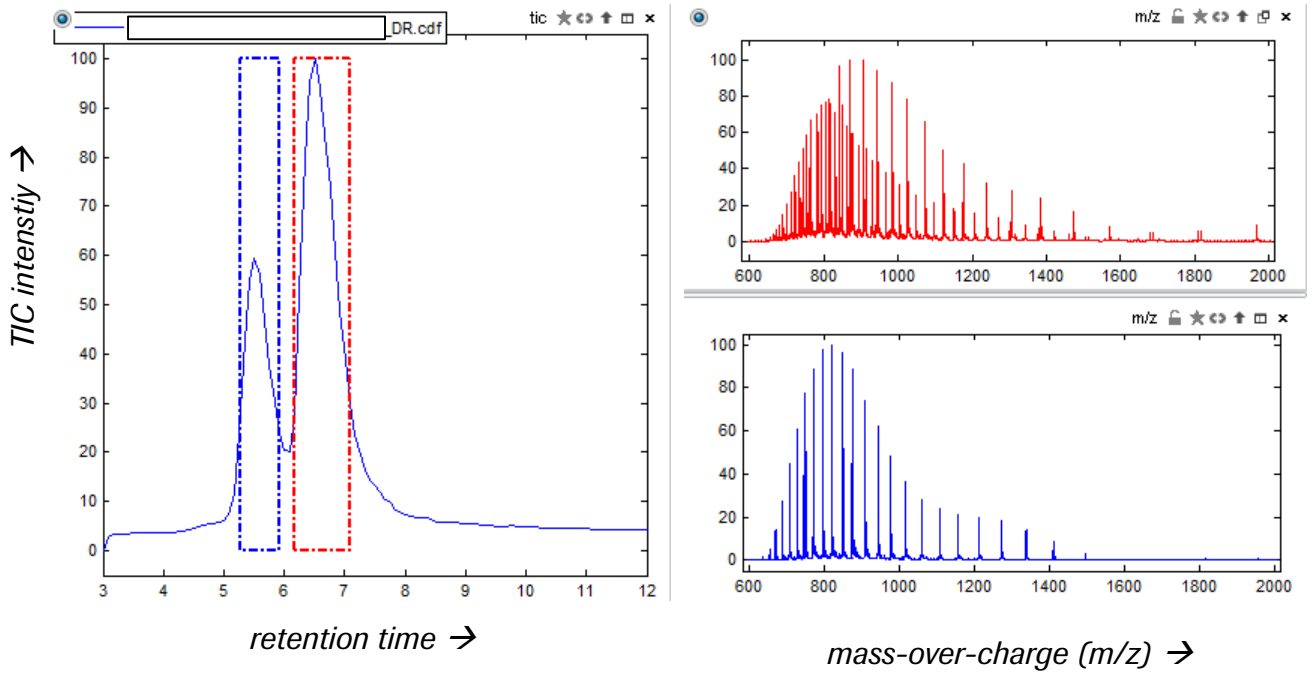
Introducing Roche's virtual MS analyst …



Combine m/z spectra of TIC peaks.

Automating MS data evaluation

Introducing Roche's virtual MS analyst …

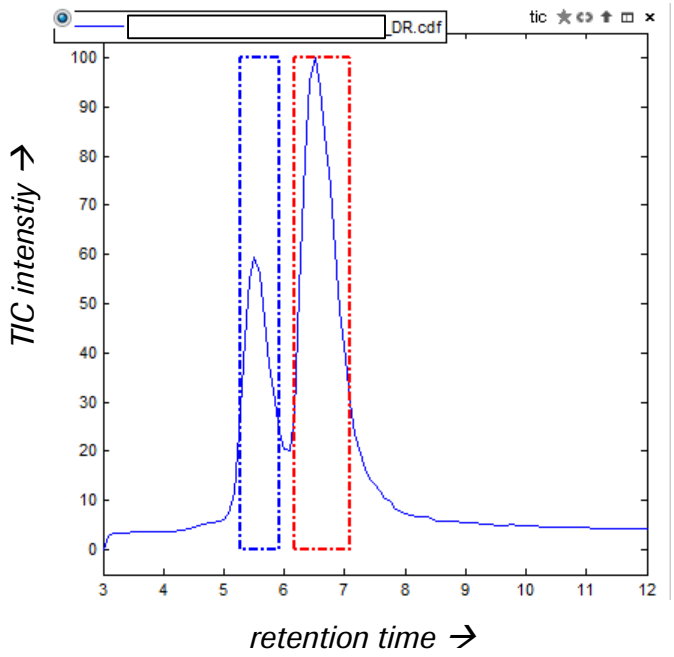


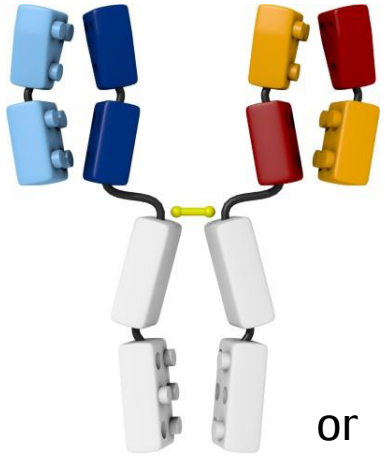
LC Peak	Mass	Abundance
1 (blue)	23338	20
1	23940	15
2 (red)	145624	100
2	145752	25
2	98345	20

Deconvolute and get a list of masses with their abundance (by ion counts).

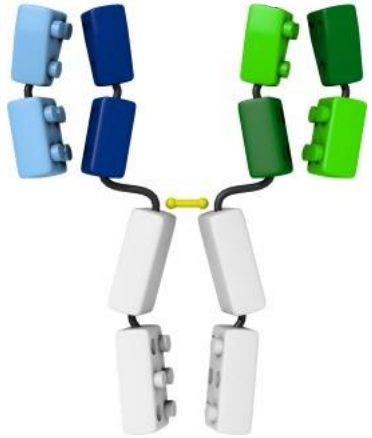
Automating MS data evaluation

Without annotations an automated ID assessment is impossible.





or



LC Peak	Mass	Abundance
1 (blue)	23338	20
1	23940	15
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Does any of these masses confirm ID?

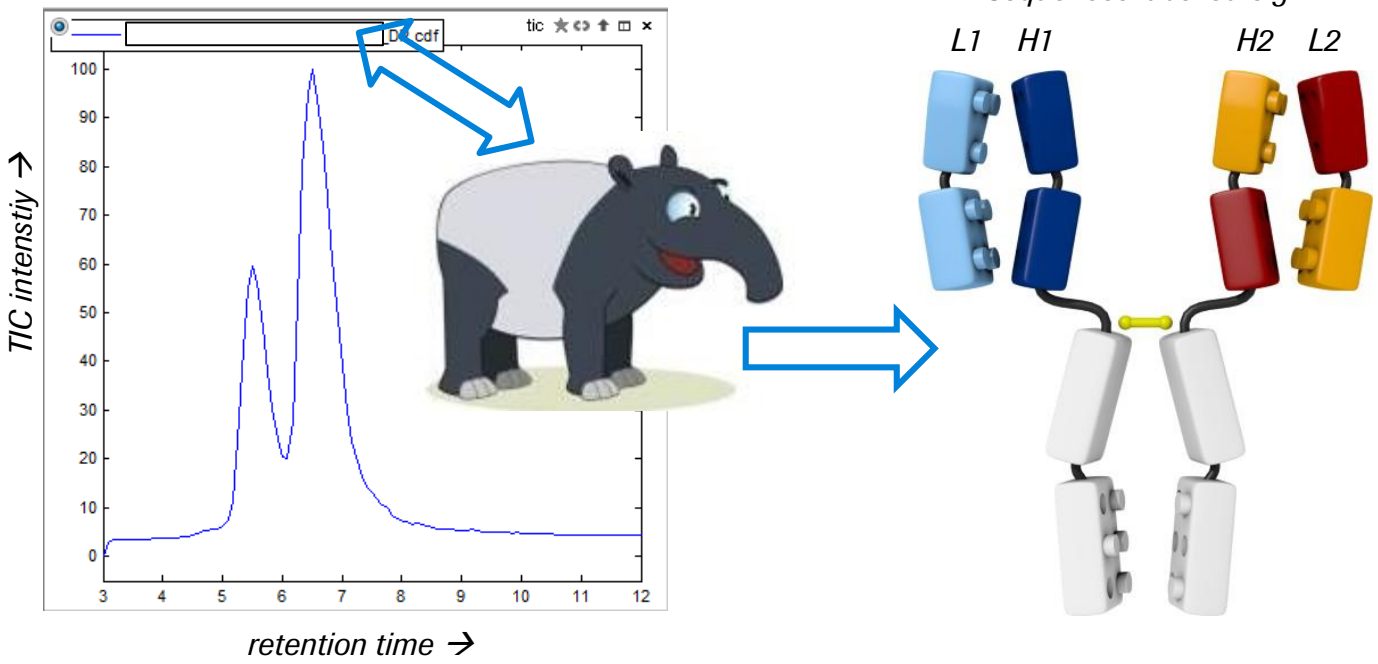
Automating MS data evaluation

Giving the virtual MS analyst some human experience

Automating MS data evaluation

Adding human MS analyst's reasoning

File name contains sample ID and preparation info



Sequences labelled e.g.

Knowing the desired main product enables deducing potential side products.

Automating MS data evaluation

Adding human MS analyst's reasoning

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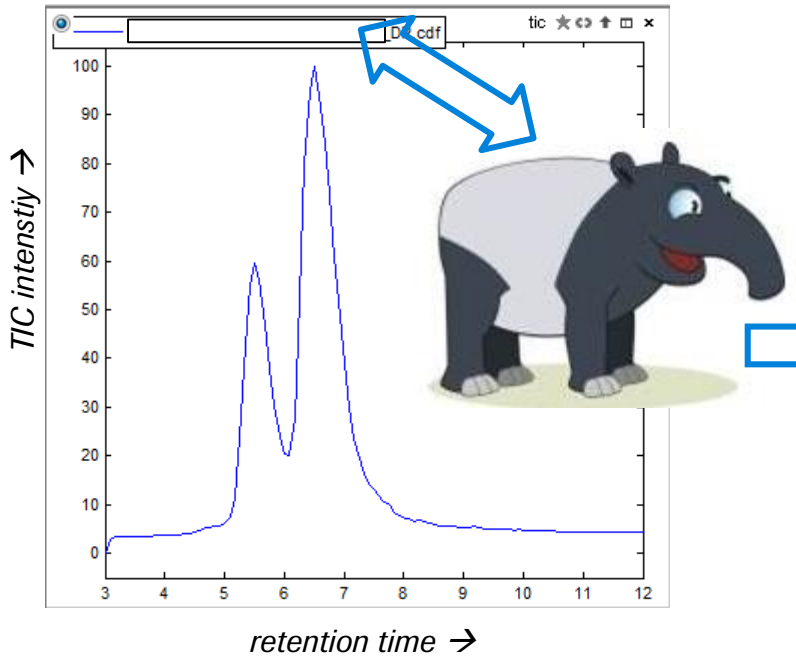
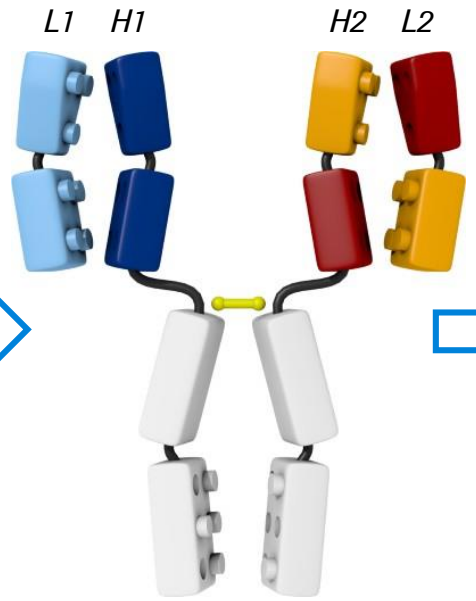
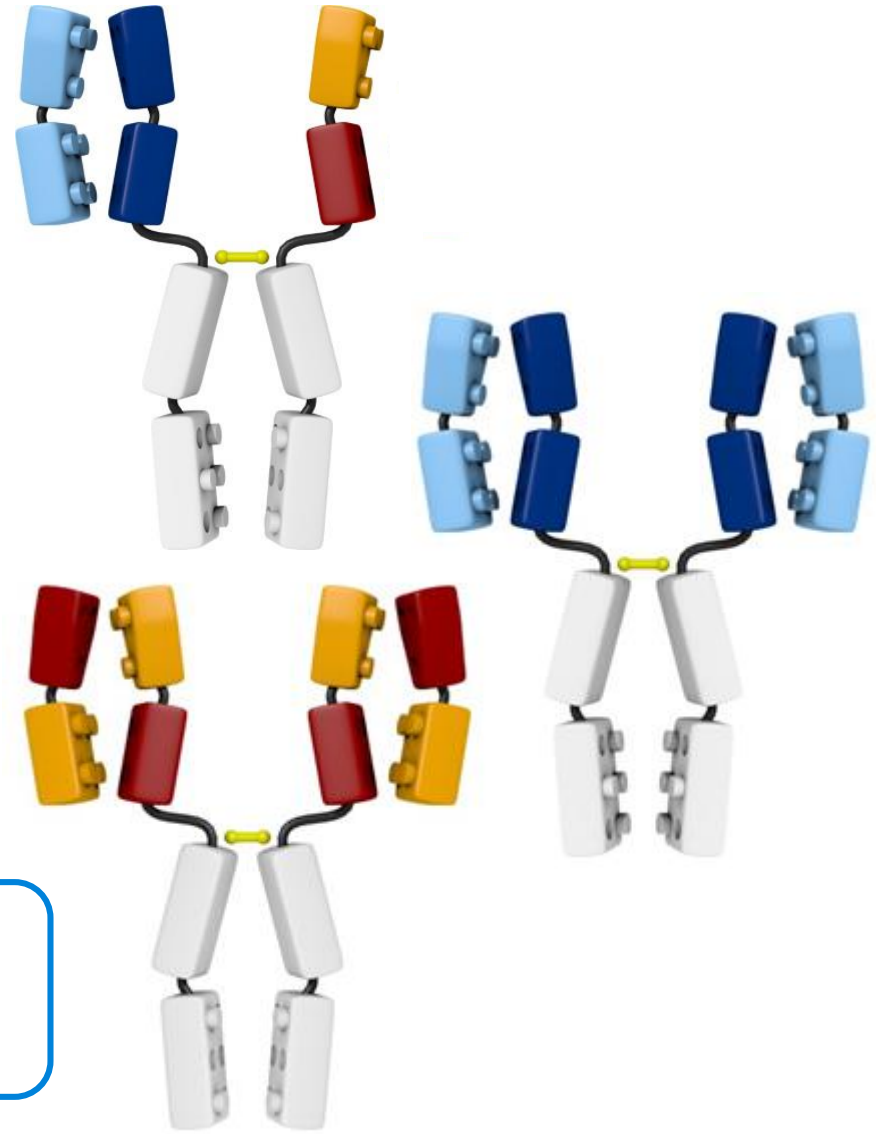




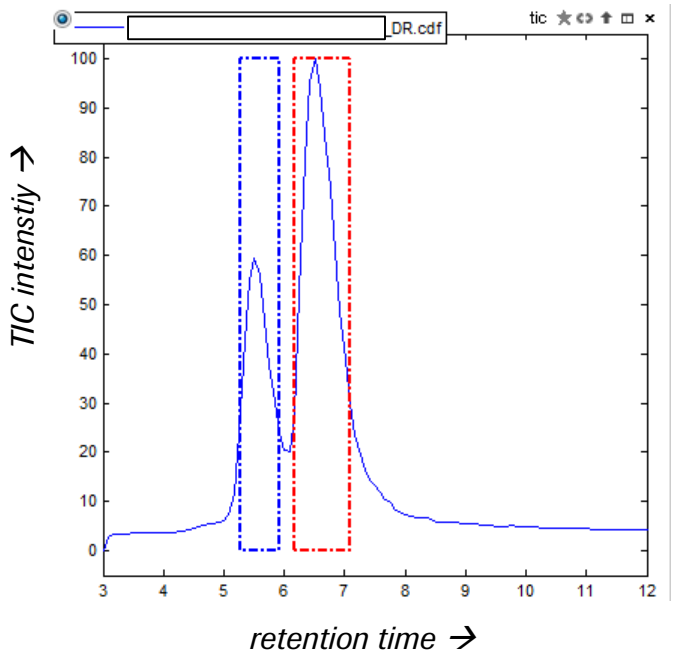


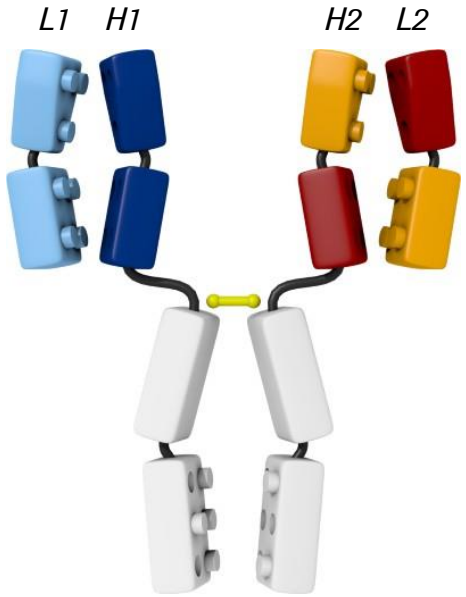
Use main product and potential side product masses for annotation.

Automating MS data evaluation

Educated virtual MS analyst provides annotations for observed masses.

File name contains sample ID and preparation info





LC Peak	Mass	Abundance	Annotation
1 (blue)	23338	20	L1
1	23940	15	L2
2 (red)	145624	100	L1 H1 H2 L2
2	145752	25	… + Lysine
2	98345	20	H1 H2

Automating MS data evaluation

Result annotations enable condensed reports including consistency checks.

LC Peak	Mass	Abundance	Annotation
1	23338	20	L1
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2	145752	25	
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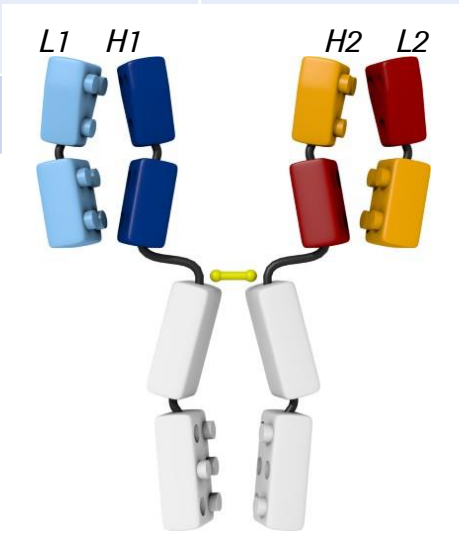
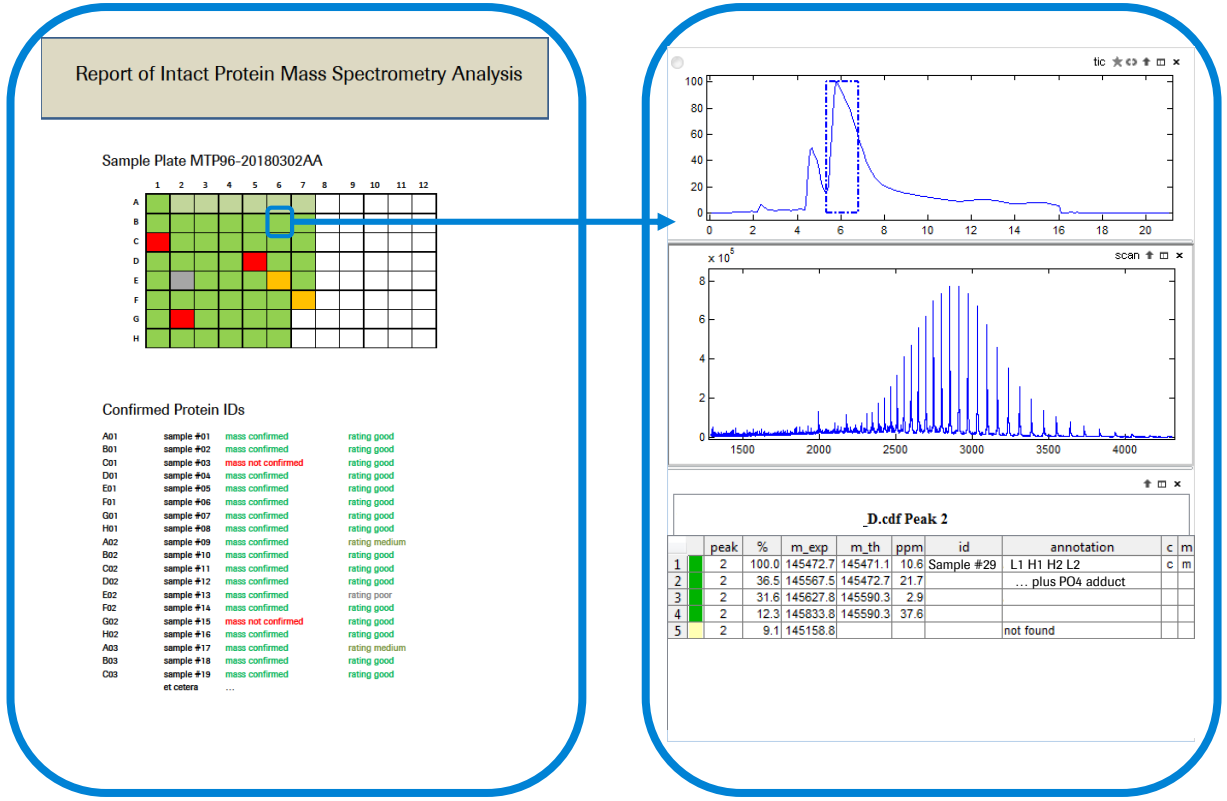


Automating MS data evaluation

Results can easily be traced back to underlying MS data for analyst review.



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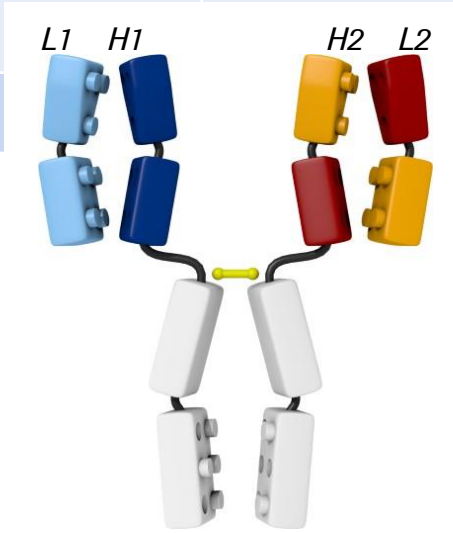
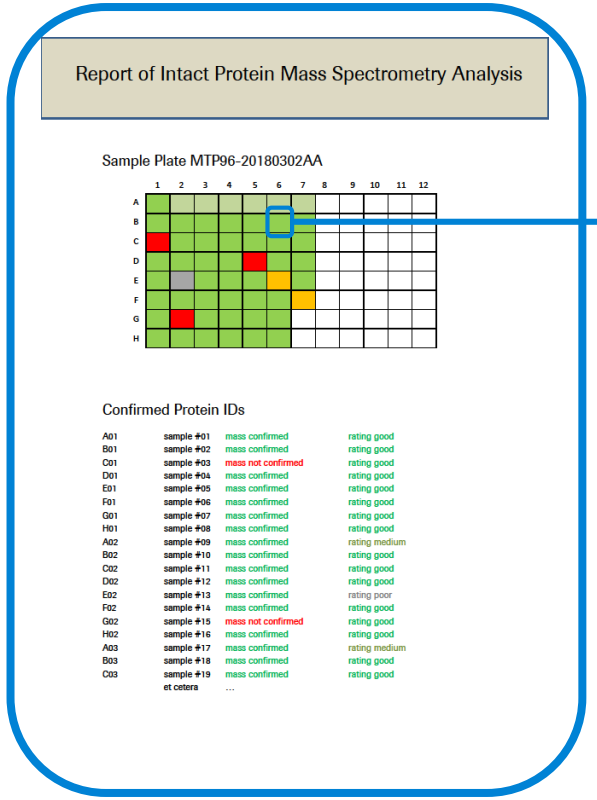
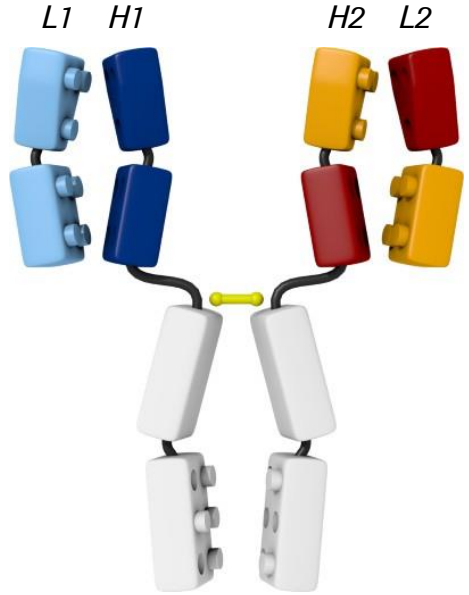


Automating MS data evaluation

Result annotations are rapidly reviewed and finalized for export to ELN.





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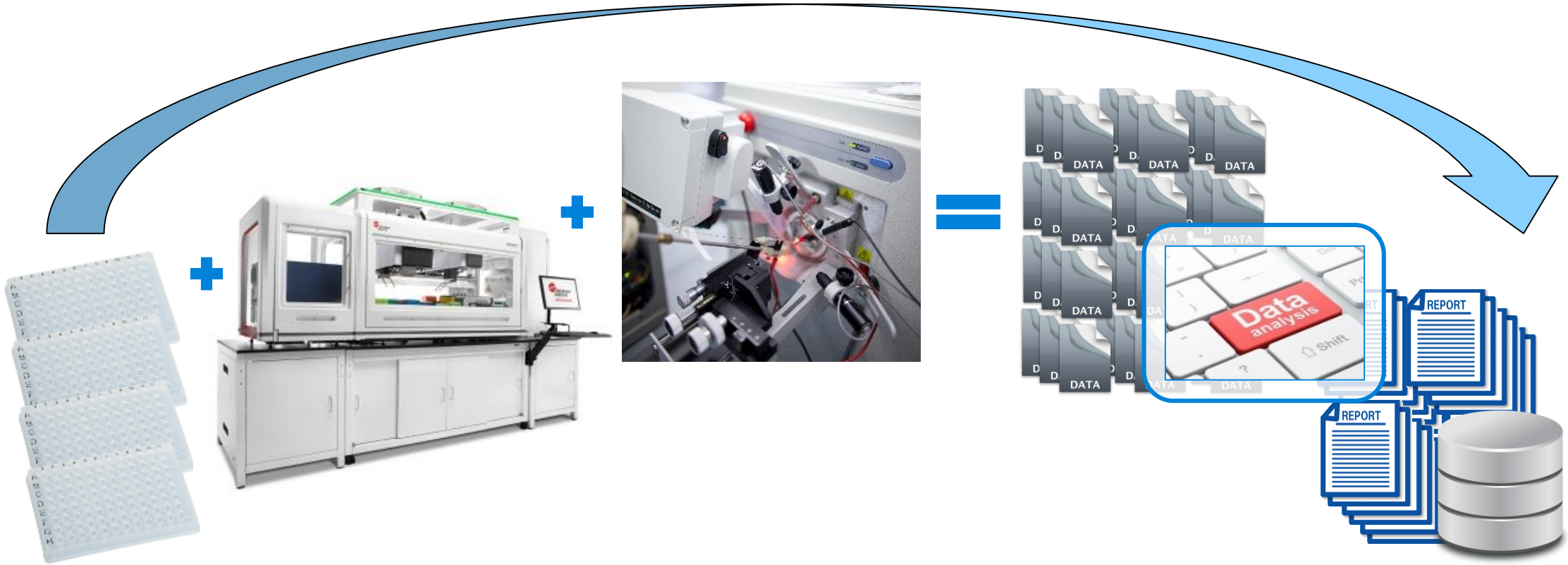
2	145624	100	L1 H1 H2 L2
2	145752	25	… + Lysine
2	98345	20	H1 H2

… and that's it.

Automation in mass spectrometry: sample-to-report

… for protein binder and format screening workflows: ***done.***



Samples — Sample preparation — LC-MS experiment — Data evaluation — Reports

Automation in mass spectrometry: sample-to-report

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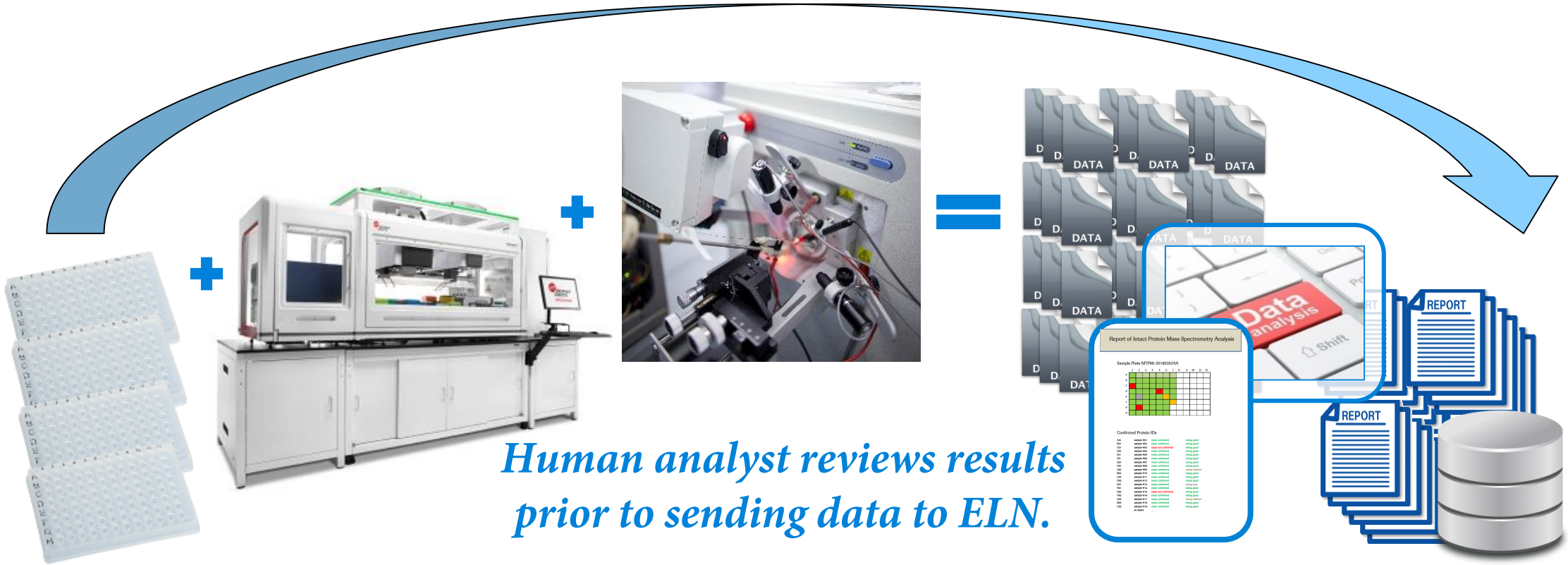

Human analyst reviews results prior to sending data to ELN.

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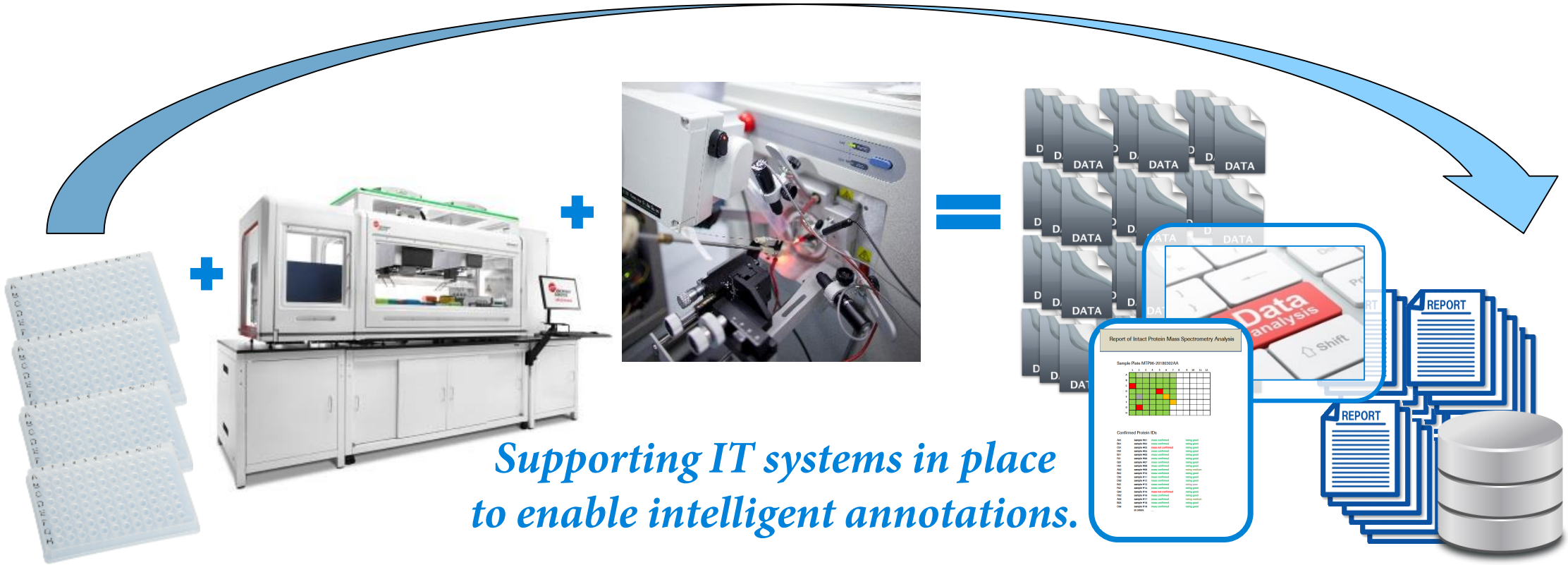
Automation in mass spectrometry: sample-to-report

… for protein binder and format screening workflows: ***done.***



Supporting IT systems in place to enable intelligent annotations.

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Queue of automation workflow candidates

Beyond support of binder and protein format screening

Extensions and enhancements of the current workflow:

- Cell line and early bioprocess development
- Improvements for protein-aware annotation engine
- CDR-focussed peptide maps (developability screening)

- Introduction
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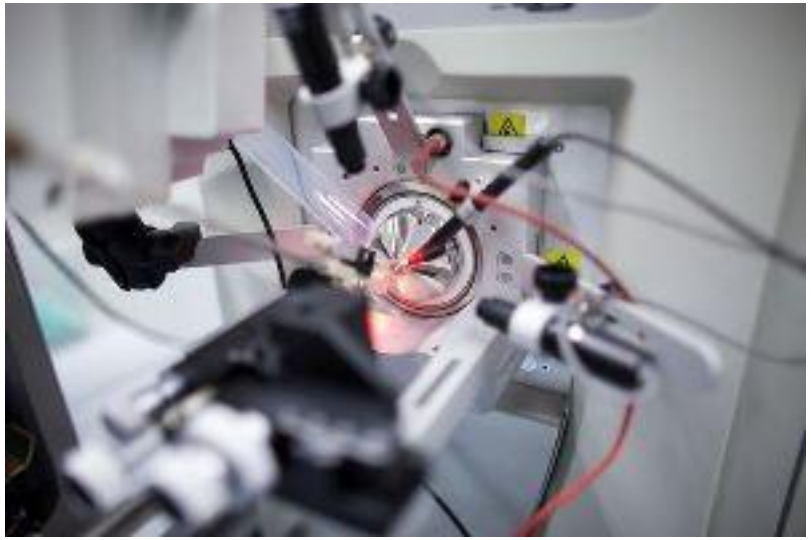
Concluding remarks

- Automation of intact protein LC-MS for binder and format screening is feasable.
- Protein information database is essential for tuning sample preparation, MS data acquisition setup as well as enabling automated annotation of result masses.
- Robustness of result annotations is linked to the signal-to-noise ratio of MS spectra.



Acknowledgements

Roche Pharma Early Research and Development

Mass spectrometry department:
Miroslav Nikolov**,** Urs Hanke, Manuel Endesfelder
Theresa Kober, Marco Böttger, Felipe Albrecht
Annette Vogt

Research IT (pREDi) and Digitalization leads:
Martin Bader, Wolfgang Paul,
Rudolf Kinder, Dominik Weitl, Angelika Fuchs

Protein modeling (lego sketches):
Alex Bujotzek

Roche Innovation Center Munich/Penzberg

Doing now what patients need next